Draft genome sequence of Cronobacter sakazakii GP 1999, sequence type 145, an epiphytic isolate obtained from the tomato's rhizosplane/rhizosphere continuum

Chase, H R ; Eberl, L ; Stephan, Roger ; Jeong, H J ; Lee, Chaeyoon ; Finkelstein, S ; Negrete, F ; Gangiredla, J ; Patel, I ; Tall, B D ; Gopinath, G R ; Lehner, Angelika

Abstract: We present here the draft genome of Cronobacter sakazakii GP1999, a sequence type 145 strain isolated from the rhizosphere of tomato plants. Assembly and annotation of the genome resulted in a genome of 4,504,670 bp in size, with 4,148 coding sequences, and a GC content of 56.8%.

DOI: https://doi.org/10.1128/genomeA.00723-17

Originally published at:
Chase, H R; Eberl, L; Stephan, Roger; Jeong, H J; Lee, Chaeyoon; Finkelstein, S; Negrete, F; Gangiredla, J; Patel, I; Tall, B D; Gopinath, G R; Lehner, Angelika (2017). Draft genome sequence of Cronobacter sakazakii GP 1999, sequence type 145, an epiphytic isolate obtained from the tomato's rhizosplane/rhizosphere continuum. Genome Announcements, 5(31):e00723-17.
DOI: https://doi.org/10.1128/genomeA.00723-17

Draft genome sequence of *Cronobacter sakazakii* GP 1999, sequence type 145, an epiphytic isolate obtained from the tomato's rhizoplane/rhizosphere continuum

Hannah R. Chase[1], Leo Eberl[2], Roger Stephan[3], HyeJin Jeong[1], Chaeyoon Lee[1], Samantha Finkelstein[1], Flavia Negrete[1], Jayanthi Gangiredla[1], Isha Patel[1], Ben D. Tall[1] Gopal R. Gopinath[1], and Angelika Lehner[3#]

[1]Center of Food Safety and Applied Nutrition, U. S. Food and Drug Administration, USA.

[2] Department of Plant and Microbial Biology, University of Zurich, Switzerland.

[3]Institute for Food Safety and Hygiene, University of Zurich, Switzerland.

Keywords: *Cronobacter sakazakii*, ST145, Tomato rhizosphere, whole genome sequencing

[#]Address correspondence to Angelika Lehner, lehnera@fsafety.uzh.ch

Abstract.

We present the draft genome of *Cronobacter sakazakii* GP 1999, a sequence type 145 strain isolated from the rhizosphere of tomato plants. Assembly and annotation of the genome resulted in a genome of 4,504,670 bp in size, with 4,148 coding sequences, and a G-C content of 56.8 %.

Cronobacter species are food associated pathogens that cause rare but severe cases of meningitis, necrotising enterocolitis, sepsis and pneumonia in preterm and/or immunocompromised infants[1-3]. The genus comprises seven species - *C. sakazakii*, *C. malonaticus, C. turicensis, C. universalis, C. condimenti, C. muytjensii and C. dublinensis*, all capable of infecting humans, with the exception of *C. condimenti* [4,5]. *Cronobacter* have been isolated from a variety of environmental sources like soil, household dust and powdered infant formula production lines, as well as from fruits, vegetables, herbs, cereals, grains [6-8], and it has also been isolated from lemon tree, wheat, rice and soybean plant rhizosplane/rhizosphere continuums [9-12].

Several lines of evidence suggest an environmental origin for *Cronobacter* with plants as ancestral econiche promoting the diversification of this genus [13, 14]. In this report, we are presenting the genome sequence of *C. sakazakii* GP1999 - originally isolated in 1999 from the roots of a *Lycopersicon esculentum* (tomato) plant by Schmid *et al.* [13].

GP1999 genomic DNA was subjected to whole genome sequencing (WGS) using the MiSeq platform (Illumina, San Diego, CA, USA), and a Nextera XT library kit. *De novo* assembly with CLC Genomics Workbench version 7.0 (CLC bio, Aarhus, Denmark) resulted in a genome of 4,504,670 bp, with 22 contigs, and a G-C content of 56.8 %. The genome was annotated using the RAST annotation server and 4,148 CDS were identified [15,16]. *Cronobacter* MLST website (http://pubmlst.org/cronobacter/) showed that it belonged to the sequence type 145 [17].

The strain harbours a pESA3/pSP291-like plasmid, which was found by comparison of the genome assembly with whole-genome sequences of strains *C. sakazakii* BAA-894 (NC_00978) and *C. turicensis* z3032 (NC_01328) and confirmed by PCR analysis. However, pESA2-like and pCTU-3 plasmid replicons were not detected by PCR [18].

Other mobilome genes found in the assembly include a total of eleven integrase/transposase genes, eight genes encoding unspecified mobile element proteins, and

63 genes encoding phage-associated proteins. Notably, a gene encoding resistance to the antibiotic fosfomycin, was found downstream of a transposase.

Other genes identified in GP1999 include virulence-associated genes encoding protein MsgA and factors VirL and MviM [15,16], multidrug resistance efflux pump-related genes belonging to *acr*AB operon, RND family, MFS superfamily and tripartite systems. Additionally, genes encoding heavy metal resistance to copper-, organic hydroperoxide, fusaric acid, and tellurite were found. Interestingly, an arsenic resistance operon repressor gene was identified downstream to an arsenic efflux pump operon and an arsenate reductase. An albicidin (a phytotoxin that blocks DNA gyrase in chloroplasts) resistance protein [19] was also observed. Furthermore, GP1999 contains a 16,771 bp operon encoding a xylose utilization pathway, supporting the hypothesis that plants represent the ancestral econiche of *Cronobacter* spp.

To the best of our knowledge, this is the first genome of a plant isolate of *C. sakazakii* being reported. The availability of the GP1999 genome will enable comparison with other genomes of *C. sakazakii* strains, thereby providing better insights into genetic features linked to plant association and possibly the natural history of this important foodborne pathogen.

Nucleotide sequence accession numbers.

The whole genome sequence of *C. sakazakii* GP1999 has been submitted to NCBI under the *Cronobacter* GenomeTrakr FDA-CFSAN bioproject number PRJNA258403, Accession#: NHTW00000000. The version described in this paper is version NHTW01000000.

Acknowledgments.

We thank the student internship programs of the International Offices of Kyungpook National University, Daegu, and Gachon University, Gyeonggi, Republic of Korea for sponsoring student interns: HJJ and CYL, respectively. We thank the University of Maryland,

Joint Institute for Food Safety and Applied Nutrition (JIFSAN) for supporting JIFSAN interns SF and FN. We also thank the Oak Ridge Institute for Science and Education of Oak Ridge, Tennessee for sponsoring research fellow HRC.

Funding information.

Funds supporting this work were obtained internally through U.S. FDA appropriations and this research was also funded in part by the University of Maryland JIFSAN Program through a cooperative agreement with the FDA, #FDU001418.

References.

1. Tall BD, Chen Y, Yan QQ, Gopinath GR, Grim CJ, Jarvis KG, et al. 2014. *Cronobacter*: an emergent pathogen a using meningitis to neonates through their feeds. Sci Prog. 97:154-172. PMID: 25108996.

2. Hunter CJ, Petrosyan M, Ford HR, Prasadarao NV. 2008. *Enterobacter sakazakii*: an emerging pathogen in infants and neonates. Surg Infect. 9:533-539. http://online.liebertpub.com/doi/abs/10.1089/sur.2008.006.

3. Osaili TM, Shaker RR, Al-Haddaq MS, Al-Nabulsi AA, Holley RA. 2009. Heat resistance of *Cronobacter* species (*Enterobacter sakazakii*) in milk and special feeding formula. J Appl Microbiol. 107:928-935. http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2672.2009.04271.x/abstract;jsessionid=034C2630C6E7AE98091CA0ECB9AF95FE.f04t04.

4. Iversen C, Mullane N, McCardell B, Tall BD, Lehner A, Fanning S, et al. 2008. *Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii* and proposal of *Cronobacter sakazakii* gen. nov., comb. nov., *Cronobacter malonaticus* sp.

nov., *Cronobacter turicensis* sp. nov., *Cronobacter* muytjensii sp. nov., *Cronobacter dublinensis* sp. nov., *Cronobacter* genomospecies 1 and of three subspecies, *Cronobacter dublinensis* subsp. *dublinensis* subsp. nov., *Cronobacter dublinensis* subsp. *lausannensis* subsp. nov. and *Cronobacter dublinensis* subsp. *lactaridi* subsp. nov. Int J Syst Evol Microbiol. 58:1442-1447. http://ijs.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.65577-0#tab2.

5. Joseph S, Cetinkaya E, Drahovska H, Levican A, Figueras MJ, Forsythe SJ. 2012. *Cronobacter condimenti* sp. nov., isolated from spiced meat and *Cronobacter universalis* sp. nov., a species designation for *Cronobacter* sp. genomospecies 1, recovered from a leg infection, water and food ingredients. Int J Syst Evol Microbiol. 62:1277-1283. http://ijs.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.032292-0#tab2.

6. Khan AA, Jones RA, Cerniglia CE. 1998. Rapid method for the detection of genetically engineered microorganisms by polymerase chain reaction from soil and sediments. J Ind Microbiol Biotechnol. 20:90–94. doi: 10.1038/sj.jim.2900489.

7. Kandhai MC, Reij MW, Gorris LGM, Guillaume-Gentil O, van Schothorst M. 2004. Occurrence of *Enterobacter sakazakii* in food production environments and households. Lancet. 363:39–40. doi:10.1016/S0140-6736(03)15169-0.

8. Friedemann M. 2007. *Enterobacter sakazakii* in food and beverages (other than infant formula and milk powder). Int J Food Microbiol. 116:1–10.

9. Gardner JM, Feldman AW, Zablotowicz RM. 1982. Identity and behavior of xylem-residing bacteria in rough lemon roots of Florida citrus trees. Appl Environ Microbiol. 43:1335–1342.

10. Forlani G, Mantelli M, Branzoni M, Nielsen F, F. Favilli F.1995. Differential sensitivity of plant-associated bacteria to sulfonylurea and imidazolinone herbicides. Plant Soil. 176:243–253. doi:10.1007/BF00011788.

11. Yang HL, Sun XL, Song W, Wang YS, Cai MY. 1999. Screening, identification and distribution of endophytic associative diazotrophs isolated from rice plants. Acta Bot Sin. 41:927–931.

12. Kuklinsky-Sobral J, Araujo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL. 2004. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol. 6:1244–1251. doi: 10.1111/j.1462-2920.2004.00658.

13. Schmid M, Iversen C, Gontia I, Stephan R, Hofmann A, Hartmann A, Jha B, Eberl L, Riedel K, Lehner A. 2009. Evidence for a plant-associated natural habitat for *Cronobacter* spp. Res Microbiol.160:608-14. doi: 10.1016/j.resmic.2009.08.013.

14. Grim CJ, Kotewicz ML, Power KA, et al. Pan-genome analysis of the emerging foodborne pathogen *Cronobacter* spp. suggests a species-level bidirectional divergence driven by niche adaptation. BMC Genomics.14:366. doi:10.1186/1471-2164-14-366.

15. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.

16. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F, Stevens R. 2014. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res. 42: D206-D214. http://nar.oxfordjournals.org/content/42/D1/D206.long.

17. Jolley KA and Maiden MCJ. 2010. BIGSdb: Scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics 11:595.9. doi: 10.1186/1471-2105-11-595.

18. Franco AA, Hu L, Grim CJ, Gopinath G, Sathyamoorthy V, Jarvis KG, Lee C, Sadowski J, Kim J, Kothary MH, McCardell BA, Tall BD. 2011. Characterization of putative virulence genes encoded on the related RepFIB plasmids harbored by *Cronobacter* spp. Appl Environ Microbiol. 77:3255–3267. http://dx.doi.org/10.1128/AEM.03023-10.

19. Hashimi, SM, Wall, MK, Smith, AB, Maxwell, A, Birch RB. 2007. The phytotoxin albicidin is a novel inhibitor of DNA gyrase. Antimicrob Agents Chemother. 51:181–187. doi:10.1128/AAC.00918-06.